CLINICAL GENETICS IN CARDIOLOGY

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The recent and rapid development of molecular genetics in cardiovascular diseases has created a new understanding of their pathogenesis and natural history, and also new possibilities for the diagnosis of these genetic disorders through genetic testing. This has induced new expectations, and new demands, from both families and physicians regarding genetic counselling, DNA testing and application of this knowledge in clinical practice. A new task for cardiologists is therefore to integrate these data in order to give the most relevant information to the patients and the relatives, to discuss genetic testing, and to use the data to optimise the management of the family. At the same time the various impacts of genetic management such as psychological, social, ethical and legal issues should be recognised, anticipated and taken into account. This is possible through a close collaboration between cardiologists and members of other medical or non-medical disciplines such as geneticists, genetic counsellors, psychologists, and molecular biologists.

The present paper focuses on some of the practical issues the cardiologist may be facing in the context of a monogenic disorder.

GOALS OF GENETIC COUNSELLING

Genetic counselling has been defined as a communication process which deals with the human and psychological problems associated with the occurrence, or the risk of occurrence, of a genetic disorder in a family.[1] The general aim is to serve the interest of the family, either patients or relatives, and the primary responsibility is to provide information as accurately as possible. The process involves an attempt to help the individual or the family to reach several objectives: (1) understand the medical facts, including the diagnosis, the probable course of the disorder and the available management; (2) appreciate how heredity contributes to the disorder and, after reviewing the family history and the pedigree analysis, determine the risk of recurrence in specified relatives; (3) understand the options for dealing with the risk of recurrence and choose the course of action which seems appropriate to them; (4) make the best possible medical and psychosocial adjustment to the recognition of a potentially heritable disorder in the family.[2] Some of these objectives might be reached through molecular diagnosis, and the individual should be counselled before the test, regarding the potential benefits but also the limitations and sometimes the potential negative effects.

Whether genetic counselling should be performed by cardiologists or by clinical geneticists, or by specifically trained non-medically qualified health care providers such as genetic counsellors, is still a matter of debate, and may vary from country to country. In a recent survey about hypertrophic cardiomyopathy in the Netherlands, cardiologists expressed their wish to inform the patients themselves and request DNA testing as their sole responsibility for *symptomatic patients*, whereas most geneticists considered a request for DNA testing in *asymptomatic relatives* as their exclusive responsibility.[3] In fact both specialties are complementary and should work together in close collaboration.

BASIC CONCEPTS OF CLINICAL GENETICS REVISED BY MOLECULAR GENETICS

Inheritance

It is first necessary to determine the precise mode of inheritance in a given family before determining the risk of recurrence in relatives (fig 1). This step may be difficult in clinical practice, even after careful pedigree analysis. Only a male-to-male transmission can affirm an autosomal dominant inheritance, whereas other situations are often also compatible with X-linked or mitochondrial inheritance (when the disease is transmitted through the mother). Moreover, molecular genetics has taught us that various modes of inheritance might be encountered (table 1),[4–10] even for a given cardiac disorder (such as X-linked and autosomal dominant in non-compaction of myocardium, or autosomal recessive and autosomal dominant in arrhythmogenic right ventricular cardiomyopathy). In other respects, classical mendelian mode of inheritance might be more complex than previously thought. X-linked cardiac diseases, such as

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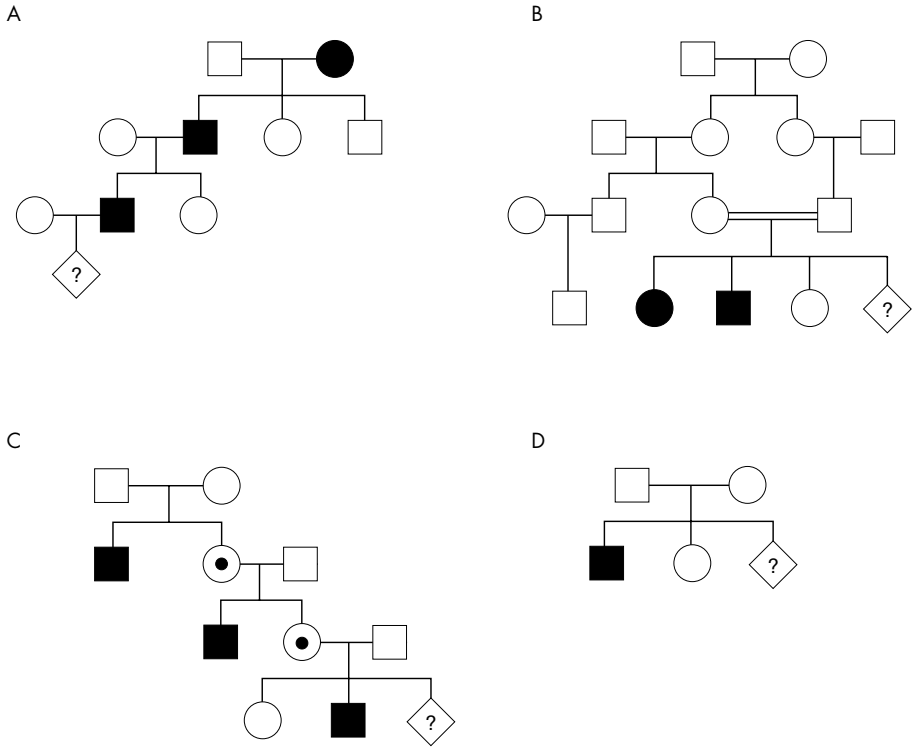

Figure 1 Mode of inheritance in monogenic disorders and risk of recurrence. Filled symbol: phenotypically affected subject; empty symbol: unaffected subject; circle: woman; square: man. ''?'' is related to the risk of being carrier of the mutation for a future child. (A) The risk is 50% in this pedigree with an autosomal dominant inheritance. (B) The risk is 25% if the inheritance is autosomal recessive. (C) The risk is 50% for future boys (and 50% for being carrier in future girls) in this pedigree with an X-linked inheritance. (D) The inheritance is very difficult to characterise from the pedigree analysis only. If the mutation is known and heterozygous in the male patient with an autosomal dominant disease, then the risk of being a carrier of the mutation for a future sibling is 50% when the mutation is transmitted from one of the two parents; however, the risk for the future child is zero if the male patient has a de novo mutation.

Fabry disease or Duchenne myopathy, were considered to be recessive, but females may develop the disease with possible severe cardiac dysfunction (although usually later in their life). In addition, an autosomal dominant disease may present itself atypically—an isolated case with a de novo mutation. Such cases are increasingly recognised in various diseases, such as hypertrophic cardiomyopathy (which is genetically heterogeneous with frequent independent mutational events and ''private'' mutations), or in the CATCH-22 syndrome (where up to 90% of 22q11 deletions are de novo mutations). Here the heterozygous mutation is observed in an apparently isolated patient but is not transmitted by one of the two parents as the mutation is absent in the two parents and paternity has been confirmed. The risk of transmitting the disease to offspring is similar to the conventional situation but the risk of transmitting the disease to the siblings of the patient is very different (considered to be null, as compared to 50% in the conventional situation). In very rare de novo mutations, however (such as long QT syndrome or hypertrophic cardiomyopathy), the risk of recurrence in the siblings was not null because of a germline mosaicism (the mutant allele was present in one of the parents but only in a small percentage of cells or in some tissues).

Penetrance

The penetrance of a mutation is the percentage of mutation carriers who express the phenotype—that is, have developed the cardiac disease on cardiac examination (with or without symptoms). The concept is important as it refers to the lifetime risk of developing the disease for a relative who carries the mutation. When the penetrance is complete (100%) it means that all mutation carriers (will) develop the cardiac disease. In contrast, some mutation carriers may not develop the disease when the penetrance is established to be incomplete. For most autosomal recessive cardiac diseases (such as long QT/Jervell-Lange-Nielsen or arrhythmogenic right ventricular cardiomyopathy (ARVC)/Naxos diseases) the penetrance is often complete, whereas the penetrance is usually estimated to be incomplete in autosomal dominant disease. In fact, recent molecular analyses have demonstrated that penetrance was age-related in autosomal dominant cardiac diseases, rather than incomplete. In hypertrophic cardiomyopathy (HCM) the penetrance is estimated to be incomplete by 30 years of age (P = 50–80%) but nearly complete (P = 90–95%) by 50–60 years of age.[4] For other diseases, or for some specific genes, the penetrance is estimated to be low (Brugada syndrome, some long QT mutations), but prospective long term follow-up is required to definitely confirm the low penetrance.[5 6]

Variable expressivity

Inheritance of most monogenic cardiac diseases observed in teenagers and young adults is autosomal dominant, and the disorders are typically characterised by a large variable cardiac expression of the disease in regard to the age at onset, the degree of symptoms, and the risk of complications. Inter-familial expressivity might be due, at least in part, to differences in the underlying gene or mutation, as genetic heterogeneity is a common rule in such cardiac diseases. A few phenotype–genotype correlations have been observed until now. In the long QT syndrome (LQT), correlations were observed between the three most frequent genes (KCNQ1, HERG, SCN5A) and the specific triggers of arrhythmia/syncope (physical exercise, auditory stimuli, rest/sleep) or the ECG patterns.[5 6] In HCM, mutations in the TNNT2 gene and some mutations in the MYH7 gene are associated with a high risk of premature sudden death, whereas mutations in the MYBPC3 gene are associated with a delayed onset and a more benign evolution.[4] Apart from inter-familial variability, there are also large differences between relatives within a given family (intra-familial variability), all of which carry the same causal mutation. This suggests a role for additional factors which may modulate the phenotype of the disease. These

Table 1 The most frequent monogenic diseases in cardiology and main genes that may be analysed for medical purposes

Disorders	Inheritance	Main genes
Long QT syndrome (LQT)	AD	KCNQ1, HERG, SCN5A
	AR (+ deafness)	KCNQ1
Brugada syndrome	AD	SCN5A
Catecholergic VT	AD	RYR2
	AR	CASQ2
Hypertrophic cardiomyopathy (HCM)	AD	MYBPC3, MYH7, TNNT2, TNNI3, MYL2, TPM1
	AD (+ WPW)	PRKAG2
Dilated cardiomyopathy (DCM)	AD	MYH7
	AD (+ CD or myopathy)	LMNA
	X-linked	Dystrophin
Restrictive cardiomyopathy (RCM)	AD	TTR (transthyretin), desmin, TNNI3
	AR	HFE
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	AD	Plakophilin2, desmoplakin, RYR2
	AR (Naxos/Carvajal disease)	Plakoglobin, desmoplakin
Non-compaction myocardium	AD	ZASP
	X linked	G4.5
Familial hypercholesterolaemia	AD	LDL receptor, apolipoprotein B
Marfan disease	AD	Fibrillin-1
CATCH-22 syndrome	AD	Del 22q11

AD, autosomal dominant; CD, conduction defect; AR, autosomal recessive; LDL, low density lipoprotein; VT, ventricular tachycardia; WPW, Wolff-Parkinson-White syndrome.

factors are not well understood but some studies reported on the role of modifier genes—that is, genetic polymorphisms which may modulate the phenotype (HCM and the renin-angiotensin-aldosterone system)—or some environmental factors (dilated cardiomyopathy favoured by pregnancy; arrhythmia favoured by certain pharmacological agents in LQT, or favoured by fever in Brugada syndrome), or by a second causal mutation (composite or double heterozygotes in HCM).

CARDIOLOGICAL SCREENING IN RELATIVES

Rationale

One of the major goals of genetic counselling is to determine which relatives are at risk of inheriting the genetic disease and therefore to propose to them a medical surveillance in order to guide preventive or therapeutic actions. Indeed the cardiac disease may be diagnosed in asymptomatic relatives through a non-invasive cardiac examination. The practical implications vary according to the diseases: a pharmacological agent might be prescribed (such as angiotensin-converting enzyme (ACE) inhibitors in dilated cardiomyopathy, β blockers in LQT syndrome, even in asymptomatic patients), or a list of drugs to avoid can be given (such as in LQT syndrome); a regular medical follow-up will be organised in all cases. Such cardiological screenings are clearly indicated by some academic societies or expert consensus reports.[11 12]

Stepwise screening

The relatives that are considered at significant risk (according to the mode of inheritance and the relationship with a patient) will be offered a cardiological examination (for example, all first degree relatives in autosomal dominant diseases, as the risk of inheritance is 50%). When the disease is diagnosed in a relative, then subsequent screening will be proposed to the relatives of this newly diagnosed patient.

Serial screening

Because of the age-related penetrance of most cardiac genetic diseases (except autosomal recessive ones, or some particular autosomal dominant diseases such as CATCH-22 syndrome), a normal initial cardiac screening does not exclude the possibility of a subsequent cardiac expression. So the at-risk relatives should have regular and repeated cardiac examinations, which have to be continued into adulthood until the age is reached when the penetrance is estimated to be nearly complete. This has been validated by some expert consensus reports[11] or suggested by some authors.[13]

GENETIC TESTING IN CLINICAL PRACTICE

Molecular strategy

Although still confined to few highly specialised molecular laboratories around the world, more and more molecular diagnoses are now performed for medical use in high quality certified laboratories, and not solely for the purpose of research studies.[14 15] They may be performed in various situations and not only for the purpose of establishing the risk of recurrence in a relative. The molecular diagnosis strategy usually begins with blood samples from a patient with an obvious form of the disease, and genes of interest are screened for a mutation by various direct (such as sequencing) or indirect (such as dHPLC) methods (table 1).[4–10 16 17] In some cardiac diseases, the analysis can be focused on one or few predominant mutations within a given gene (such as haemochromatosis/HFE gene or familial amyloidosis/TTR gene). The method is easy and the results can be given in a few days or weeks. But for most cardiac diseases, the analysis should concern all the coding sequence of the gene as the mutations can be located at full length of the genes (in LQT syndrome, Brugada, HCM, etc). Moreover, many genes might be involved in a given monogenic disease (but only one gene is involved in a given family), and the most frequent genes are sequentially analysed (table 1). The process is therefore quite long and results are usually given within months. The efficiency of the mutation screening depends on the careful characterisation of the phenotype (and the possibility of phenocopies), the complexity of the underlying molecular data (the number of genes to screen, the length of the genes to screen, the possible involvement of additional as yet unidentified genes), and the sensitivity of the molecular methods. The usual efficiency (possibility to identify a mutation in a patient with an obvious form of the disease) of a conventional mutation screening in a medical laboratory may therefore vary from very high (> 90% in haemochromatosis) to quite high (50–70% in LQT syndrome or HCM) to quite low (~20% in dilated cardiomyopathy or Brugada syndrome). Conversely, when the causal mutation is identified

Table 2 Main outcomes of predictive genetic testing in autosomal dominant disorders

	If the mutation is present	If the mutation in absent
Positive outcome	Removal of uncertainty Regular medical follow-up is organised, which will improve the prognosis In very rare cases, possible treatment to begin or drugs to avoid	Removal of uncertainty, and relief No future development of the disease, and medical follow up is no longer required No risk of transmission to offspring
Negative outcome	Anxiety because of future cardiac expression (risk of premature death) No treatment to begin at this stage in most disorders Risk of transmission to offspring	Possible "survivor" guilt
Uncertainties remaining	Recommend environmental modifications? (exercise or alcohol restriction, avoid fever) Medical costs? Insurability or professional concerns	Not always easy to affirm that the mutation identified in the probands is the cause of the disorder in the family, especially if missense mutation

in the index patient (or proband) of the family, then it is very easy and quick to determine the genetic status of relatives within this family (within a few days or weeks).

Diagnostic testing

The correct diagnosis of a cardiac disease of genetic origin usually does not require genetic testing and molecular proof before the cardiac and therapeutic management of the patient can begin. In some situations, however, molecular diagnostic testing may help the clinician to make the right diagnosis. This can be performed in the presence of borderline cardiac abnormalities, such as significant but reversible LQT after stopping the causal medication, or the discovery of mild myocardial hypertrophy in an athlete (possibly related to athlete's heart or to the onset of HCM).[4–6] Molecular tools, along with a cardiological inquest with the relatives, might offer crucial information for the management of such individuals. Even in patients with an established diagnosis of cardiac disease, diagnostic testing might help to differentiate between several genetic causes (for example, in a patient with HCM) so that specific therapy could be applied (such as in Fabry disease), or genetic counselling could be modified (X-linked inheritance in Danon disease), or specific follow-up could be recommended (risk of conduction defects for PRKAG2 gene mutations). In addition, a patient with an apparently "sporadic" form of a disease may wish to know if the disorder is of genetic origin (such as ARVC) in order to determine the risk of transmission to offspring. For all these situations, and most of the cardiac disorders, it should be borne in mind that only a positive result (identification of a mutation) is meaningful, whereas a negative result (no mutation is identified) usually does not lead to a conclusion (when there is a known genetic heterogeneity).

Prognostic testing

In the context of cardiac diseases with phenotype–genotype correlations, genetic testing might be useful for the cardiologist to better stratify the prognosis of a patient with the disease. Knowledge of the precise gene and mutation underlying the disease in a given patient could help the clinician to identify better the patients at high or low risk of cardiac death, or to identify better the responders or non-responders to a given pharmacological treatment. This would have a medical impact with a more accurate choice in the therapeutic strategy. However, only few such correlations have been described until now in cardiology, and the results still remain to be validated. In the LQT syndrome, patients with a mutation in the SCN5A gene respond less well to β blockers, and an alternative treatment might be considered.[5 6] In HCM, patients with a high risk of sudden death (mutation in the TNNT2 gene, for example) might benefit from an invasive therapeutic strategy such as a cardioverter-defibrillator.[4] In dilated cardiomyopathy (DCM) related to a mutation in the LMNA gene (lamin A/C protein), the evolution towards severe conduction abnormalities or ventricular arrhythmia, which can occur in relatives before the onset of the DCM, lead to a specific medical surveillance and a discussion about early implantation of a pacemaker and/or defibrillator.

Predictive diagnosis

When the mutation is identified in the proband of a given family, genetic testing can be proposed to the apparently healthy relatives within the family to determine their genetic status and their exact risk of evolution. Most autosomal dominant cardiac diseases are especially of concern as the penetrance is age related with a frequent delayed onset of the cardiac phenotype. In such diseases, and in the absence of a mutation, the relative will be reassured, a medical follow-up will be no longer required, and there is no risk of transmitting the disease to offspring. In contrast, in the presence of a mutation, a strict medical follow-up is required, in order to improve the therapeutic management of the disease (table 2). Additional preventive or therapeutic measures can also be discussed at this early stage of the disease, such as medical treatments (β blockers in long QT syndrome) or the need to avoid certain contraindicated drugs (in long QT or Brugada syndromes), or the need to consider environmental factors which may favour/induce the cardiac expression or complications of the disease (restricting alcohol consumption in DCM patients or physical activity in ARVD and HCM, avoiding fever in Brugada syndrome). Possible negative impacts of the predictive test should, however, be recognised and anticipated, such as the psychological burden placed on individuals upon learning they are almost certain to develop the disease later (if the penetrance is nearly complete), and the risk of transmitting the disease to offspring.[18 19] Uncertainties also remain about the confidentiality of the result, and possible insurance or professional concerns.[2] In children, the psychological impact of the test is even more important and predictive testing is often a matter of debate, especially in children less than 10 years old (except in LQT syndrome because of therapeutic implications).[20] Because of the complex medical, psychological and social implications of

Table 3 Main principles of genetic testing, especially for predictive testing

- The subject should be informed and counselled in advance (before blood sampling)
- The decision to make the test is solely the choice of the individual concerned
- Written informed consent has to be signed and retained
- There must be respect of the right to know, and the right not to know (for the subject)
- Molecular analysis should be performed in high quality certified medical laboratories
- The result of the test should be given in person to the individual
- Confidentiality should be respected so that the person can not be discriminated against in any way

predictive testing, careful consideration is required. All these aspects should be discussed before blood sampling, by trained health care providers and if possible through a multidisciplinary team, according to general well established principles of genetic counselling (table 3).[1 2 18–20] In X-linked diseases, the diagnosis of females who carry the mutation and can transmit the disease may share some of these considerations.

Prenatal diagnosis

Some couples may request prenatal diagnosis through amniocentesis or chorionic villus sampling at the beginning of the pregnancy to determine the genetic status of the fetus and discuss pregnancy termination if the mutation is present. The process might be theoretically discussed when the mutation is characterised in the parent(s) and it can be medically supported when several conditions are present: the risk of developing the disease is certain, there are no curative treatments, and the risk of premature death or severe disability is very high. Only few cardiac diseases meet these conditions. Most diseases are characterised by an incomplete (Brugada syndrome) or age related penetrance (especially in HCM), or a variable expressivity with a low absolute risk of premature death (especially in HCM), or the possibility of an efficient treatment (either pharmacological as in LQT syndrome, or a cardioverter-defibrillator as in Brugada). Prenatal diagnosis is therefore discussed only in the context of some specific diseases (such as Duchenne or Steinert myopathies, or the CATCH-22 syndrome) or severe forms with an autosomal recessive inheritance, or in particular cases (such as a premature death in a previous child). In any case, the decision to perform prenatal diagnosis is particularly difficult and should be made after case-by-case discussion through a multidisciplinary team, according to a consensus that has to be reached between the parents and the medical team. Other options should also be discussed, such as adoption, artificial insemination using donated gametes, pre-implantation genetic diagnosis, remaining childless, or the acceptance of having a child with a genetic disorder.

CONCLUSION AND PERSPECTIVES

Recent surveys suggest that an important gap already exists between the rapidly increasing genetic knowledge of cardiac diseases and the medical applications in practice. The objective is to now fill the gap, integrating genetic counselling, and the possibility of genetic testing, in the management of patients and their relatives. One of the primary goals is to inform a patient about the risk of transmitting the disease within the family, and to organise appropriate screening and follow-up of the relatives. The discussion of genetic testing requires careful consideration as various medical, psychological, ethical, social and legal aspects must be taken into account. This can be achieved through close collaboration between different specialties and trained health care providers. A high level of expertise is mandatory for some specific situations, such as predictive or prenatal testing. We enter the era of multidisciplinary teams. The establishment of organisational structure coordinating clinical, laboratory and research work has also been proposed, to ensure a quick translation of the knowledge into medical applications.[2]

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